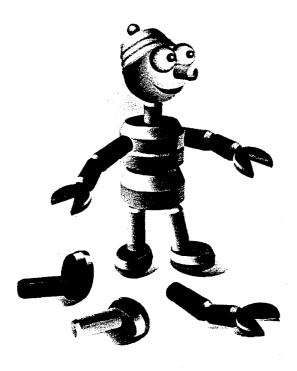
International News on Fats, Oils and Related Materials

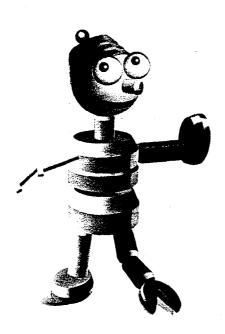
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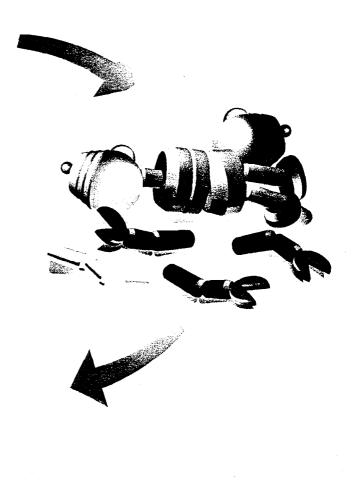


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INSTRUMENTATION

SFE—new method to measure oil content

In 1996, the AOCS Uniform Methods Committee voted approval of official method status to a world-wide collaborative study entitled "Measurement of Oil Content of Oilseeds by Supercritical Fluid Extraction (SFE)."

Seventeen laboratories in eight countries analyzed 40 samples in the SFE collaborative study. Coordinators were David L. Berner, who at that time was AOCS Technical Director, and Les Myer, who at that time was with Isco Inc. of Lincoln, Nebraska, a manufacturer of instruments including several analytical SFE units.

The new official method is Am 3-96 and complete details can be found in the 1996-1997 edition of AOCS' Official Methods and Recommended Practices.

SFE most often uses safe, inexpensive supercritical carbon dioxide in lieu of organic solvents. SFE instruments draw CO₂ out of ordinary dip tube cylinders, then heat the CO₂ and pressurize it to a superfluid state, where it retains its gas-like viscosity to penetrate solid or powdered samples very quickly, while possessing the solvating power of an ordinary liquid. Samples are placed into cartridges, and their fat or oil content is extracted in minutes, not hours, using supercritical CO₂.

Compared to classic Soxhlet (or Butt tube) extractions, the principal benefits of SFE are speed of analysis, reduction or total elimination of traditional solvents, with their attendant



Technician loads samples into Isco SFE unit for fat/oil content analysis.

purchase and disposal costs. Some SFE instruments are fully automated, sequential extractors. Others do single samples, or between 2–9 samples in parallel. Fat or oil is measured gravimetrically on an analytical balance, or can be subjected to fatty acid methyl ester analysis, as required by the U.S. Nutritional Labeling and Education Act (NLEA) (1).

The five oilseeds used in the AOCS collaborative study were soybeans, cottonseed, canola (rapeseed), sunflower, and safflower.

It should be noted that initial labo-

ratory interest in the new method comes from two directions: first, providing a method for crushing plants to measure oil content of an oilseed before or during processing. Although Am 3-96 deals with oil content of ground seeds, excellent oil recoveries also have been performed via SFE on seed components such as meal, hulls, and flakes; second, analytical laboratories need a primary method to calibrate rapid analyzers such as near-infrared reflectance (NIR) and nuclear magnetic resonance (NMR). Some rapid analyzers used for seed oil content are prone to drift, or display interferences, because the wavelengths for oil and fiber measurement are in close proximity. NIR and NMR are secondary methods and must be calibrated by a primary method employing complete extraction. Soxhlet extractions provide these data, but require several hours for a complete extraction. The new SFE method, however, is a primary calibrating method and usually requires only a 30-minute extraction of the oil. Therefore, users of rapid NIR and NMR instrumentation for oil content can calibrate much more frequently to control drift.

Confidence in this approach has



W.V. O'Farrell

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International earlier this year announced Dr. King would receive its 1997. Harvey W. Wiley Award, the organization's highest scientific award.

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Table 1
Collaborative tests on SFE analysis

	# labs after outliers deleted	# of labs deleted		Mean oil content, x (method of extraction)				
			Accepted values, n	AOCS Ac 3-44	Exhaustive extraction	AOCS Am 3-96	S _r	S _R
				(Butt-tube)	(AOCS 2-93)	SFE	-	
Commodity Soybean			•					
CO ₂	14	3	28	19.06	21.67	18.4	0.34	0.38
	13	4	26	19.35	21.89	19.3	0.58	0.58
CO ₂ + EtOH	12	3 2	24	19.06	21.67	20.5	0.43	0.77
	13	2	26	19.35	21.98	20.4	0.58	0.87
Rapeseed								
CO ₂	15	2	30	43.74	45.20	39.8	0.45	0.90
	15	2 2	30	40.29	42.70	37.7	0.67	1.03
CO ₂ + EtOH	12	3	24	43.74	45.19	43.5	0.37	1.32
	14	1	28	40.29	42.73	40.2	0.86	1.43
Cottonseed								
CO ₂	14	3	28	18.17	20.60	19.1	0.46	0.51
	14	3 3	28	17.53	20.00	18.4	0.34	0.38
CO ₂ + EtOH	12	3	22	18.17	20.61	19.7	0.17	0.53
	14	1	27	17.53	20.00	19.3	0.35	0.52
Sunflower								
CO ₂	13	4	26	38.74	41.75	38.8	0.73	0.89
	13	4	26	42.71	46.01	42.3	0.64	1.08
CO ₂ + EtOH	11	4	22	38.74	41.75	40.2	0.44	1.13
	12	3	24	42.71	46.01	43.0	0.63	1.64
Safflower								
CO ₂	13	4	26	37.99	40.06	36.9	0.83	0.93
	14	3	28	36.41	39.10	35.7	0.86	1.05
$CO_2 + EtOH$	12	3	23	37.99	40.64	38.0	0.77	1.14
-	11	4	22	39.07	36.41	37.1	0.67	1.00

 S_r = repeatability standard deviation; S_R = reproducibility standard deviation.

been shown by a major manufacturer of snack foods that purchased more than 60 SFEs for the purpose of calibrating NIR units more frequently.

The new Official Method Am 3-96 is actually a main method plus an optional "exhaustive" method requested by FOSFA (Federation of Oil, Seed, and Fats Associations) International. The initial method calls for a 30-minute extraction of oil from 2-gram sample of seed that has been ground, using only supercritical CO₂ at 7,500 psi and 100°C. Flow rate was 3.2 mL/min. Under these conditions, phospholipids are relatively insoluble in CO₂. After discussions with FOSFA International, it was felt that an alternative exhaustive method also

should be part of the 1995–96 collaborative study to allow phospholipids to be co-extracted with the seed oil. This was accomplished by pumping 15% ethanol along with supercritical CO₂, which will remove all oil and most phospholipids in 60 minutes. Flow rate was 2.0 mL/min.

Most analytical SFE instruments can be programmed to automatically mix supercritical CO₂ with modifier solvents such as 15% ethanol. Again, although Official Method Am 3-96 calls for a 30-minute extraction on beans or seeds at 100°C and 7,500 psi with neat CO₂, methods for oil extractions from meal, flakes, and hulls were optimal at 130°C with ethanol modifier mixed with supercritical CO₂.

Official Method Am 3-96 calls for direct gravimetric determination of oil content. The extracted oil is collected outside the SFE unit in a test tube filled with glass wool. The test tube is weighed before and after extractions from beans and seeds. Extracted moisture is either dissipated by the instrument's heated flow restrictor or baked off in a post-extraction drying step, if required. Although indirect (weight loss) oil content methods are possible, Taylor, King, and List (2) recommend against these due to potential water interference in the gravimetric assay.

Results using the above methods are shown in Table 1 for the SFE of the five oilseeds previously listed, for

(continued on page 1050)

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neat supercritical CO₂, and for the addition of ethanol as a modifier to the supercritical fluid which is equivocated to an exhaustive extraction. Also tabulated are the results obtained using the existing AOCS method.

Why use a 30-minute extraction with CO₂? After extensive kinetic trials performed by the project coordinator, coupled with discussions with members of the AOCS Seed and Meal Analysis Committee, it was decided that even though most of the oil was extracted in the first 10 minutes with supercritical CO₂, an additional 20 minutes of extraction provided complete oil extraction except for the phospholipid content. Also, SFE at a 30-minute time interval gave results comparable to Butt-type extractions used in AOCS Official Method Ac 3-44.

Measurement of oil in oilseeds *via* SFE is the first SFE method to be made official by AOCS or FOSFA Interna-

tional. In addition, AOAC International decided to jointly approve the AOCS method, the first SFE method approved by that organization.

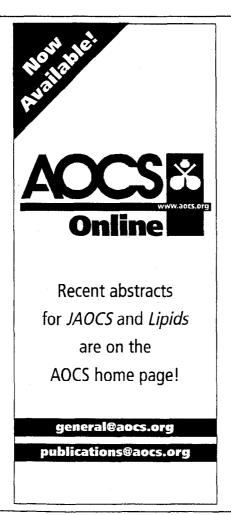
In the aforementioned SFE paper (2), corn germ oil content was measured successfully via SFE. In 1997, a novel program was instituted in West Des Moines, Iowa, by DuPont Quality Grains, a producer of "Optimum," an identity-preserved high-oil corn. Farmers for the first time are being paid premiums to produce this corn, which has an average oil content of 6.5–7.5%. Initially, corn oil content is measured at the grain elevator by NIR, calibrated by an SFE at DuPont's lab. If the grain elevator's NIR has not been calibrated by DuPont-approved calibrations, the elevator sends a sample to that firm's SFE lab whose oil analysis is then used to determine the price premium paid to the grower. One million acres of such corn were planted in 1997 in the United States; DuPont Quality Grains has said it expects to double that acreage in 1998.

It should be noted that SFE has been applied not only for the gravimetric determination of oil/fat content in seeds and foodstuffs but also has been integrated into the protocol required by the NLEA requiring fatty acid methyl ester analysis for the quantitation of total, saturated, and cis-monounsaturated fat content (1). Recent studies have shown that enzymatic conversion of fats and oils to fatty acid methyl esters can be accomplished in the presence of supercritical carbon dioxide, permitting an integrated extraction-reaction and chromatographic analysis protocol to be achieved on several different types of automated SFE instruments (3), Snyder et al. (4) recently have demonstrated that the above sequence not only is applicable to glyceride species but also can be used to determine the fatty acid content of minor lipid-containing constituents, such as phospholipids and cholesteryl esters (4).

What's down the road for characterizing oilseeds via SFE? Geneticists are currently measuring oil content of transgenic oilseeds using SFE to evaluate new strains. The Supercritical Fluid Technology Group at the National Center for Agricultural Utilization Research in Peoria (Taylor and King) have developed a promising SFE alternative to the lengthy AOCS test for "Total Fatty Acids in Soapstock." Results from this shortened analysis were presented at the AOAC International meeting held during September 1997 in San Diego.

Meanwhile, scientists at Isco, Texas A&M University, and elsewhere are collaborating on optimizing an SFE method for neutral oil and loss (NOL), presently a time-consuming AOCS method requiring ethyl ether as a solvent.

Liescheski recently has coupled SFE with infrared instrumentation for determining the total oil content in milled rice flours, the iodine number of extracted oils, and *trans* fatty acid contents in fats and oils. This analytical application of supercritical fluids (5) and a review of the determination of fat content in foods by analytical SFE by Eller and King (6) should be



consulted by persons interested in further reading on these subjects.

Supercritical fluid extraction has proven to be a costeffective, solvent elimination tool for oilseed analytical laboratories and for research labs involved with the analysis of lipid moieties. There should be a bright future for this rapidly emerging technique as laboratories seek ways to speed analyses and reduce traditional solvent consumption and disposal.

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SFE equipment

AOCS Official Method Am 3-96 (Oils in Oilseeds: Supercritical Fluid Extraction Method) specifies use of a supercritical fluid extractor capable of producing 7,500 psi and a temperature of 100°C. The extractor should be equipped with a heated restrictor (adjustable or fixed flow) capable of keeping the temperature of the evolving $\rm CO_2$ above 60°C. For extractions with $\rm CO_2+15\%$ ethyl alcohol, a modifier pump is required. For further details, see the official method.

Persons who participated in the collaborative testing leading up to adoption of the methods used SFE equipment from the following manufacturers:

Isco Inc. of Lincoln, Nebraska; Leco Corporation of St. Joseph, Michigan; and Suprex Corporation, which was acquired by Isco during 1996.

Other firms manufacturing SFE systems include: Applied Separations Inc. of Allentown, Pennsylvania; Hewlett-Packard Co. of Wilmington, Delaware; Jasco Inc. of Easton, Maryland; and Supelco Inc. of Bellefonte, Pennsylvania.

Equipment manufacturers should be able to verify whether their SFE units meet the specifications of the method.

